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Arrangement of blood vessels and their relation with adrenergic nerves in the rat mesentery

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INTRODUCTION

Lying, as it does, in a transparent sheet which can be held flat for microscopic observation, the mesenteric microvasculature has proved suitable for studies of small vessels and their reactions to experimental manipulations (Landis, 1930; Rogers, 1932; Zintel, 1936; Zweifach & Kossman, 1937; Chambers & Zweifach, 1944; Johnson & Wayland, 1967; Altura, 1971*b*). In spite of the extensive use of this preparation, especially in the rat, there has been no detailed study of its innervation with the specific histochemical methods now available. Indirect evidence from the responses of the small mesenteric vessels when a rat is deprived of oxygen suggests that the density of arterial innervation by constrictor fibres decreases towards the capillaries (Zweifach, 1961), and this assumption is strengthened by the observation that the precapillary arterioles, although they have muscular walls, do not respond to the stimulation of nerves supplying the mesentery (Furness & Marshall, 1973*a*, *b*). However, larger vessels of the microvasculature, the small arteries and terminal arterioles, are constricted by nerve stimulation, and this constriction is blocked by guanethidine or phentolamine. On the venous side, too, the smallest muscular vessels do not respond to stimulation of nerves to the mesentery. The present experiments were undertaken to determine if the distribution of nerves implied by the experiments outlined above would be confirmed by histochemical observations using the fluorescence method for localizing catecholamines.

Previous morphological studies have only partly identified the extent of the adrenergic innervation of terminal vascular beds. The early investigations were hampered by the inconstancy of silver and methylene blue staining of the smallest fibres and by the difficulty of positively identifying the structures revealed by these methods; nevertheless, it was established that arterioles and medium-sized arteries were supplied by a network of fibres at the adventitiomedial border (Stöhr, 1932; Mitchell, 1953). It was not determined how far along the arterial tree the sympathetic fibres extended or whether the capillaries were innervated (Krogh, 1922; Stöhr, 1932; Kuntz, 1953). When electron microscopical techniques and the fluorescence histochemical method for adrenergic nerves became available, it was soon demonstrated that the capillaries are not adrenergically innervated and that the arterial innervation extends at least as far as small arterioles (Lever & Esterhuizen, 1961;

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Zelander, Ekholm & Edlund, 1962; Appenzeller, 1964; Fuxe & Sedvall, 1965; Lever, Graham, Irvine & Chick, 1965; Ehinger, Falck & Sporrong, 1966; Simpson & Devine, 1966; Devine & Simpson, 1967). Except for the work of Samarasinghe (1965), on the cerebral arteries, and Rhodin (1967), on vessels in the fascia of skeletal muscle, there is still little morphological information concerning the distance that adrenergic nerves extend along the arterioles. The mesentery is supplied by nerves which arise from prevertebral ganglia and follow the superior mesenteric artery and its branches to the intestine (Mitchell, 1953). Rami from these visceral nerves provide an adrenergic innervation of large arteries and veins and at least some of the mesenteric arterioles (Falck, 1962; Devine & Simpson, 1967; Furness, 1971).

The arrangement of blood vessels in the rat mesentery has also been examined in the present investigation. The topography of part of this vasculature has been described already (Chambers & Zweifach, 1944; Zweifach, 1961), but the authors were concerned only with the capillaries, the muscular vessels from which these arise, and the venules which they join. Unlike the present work, their study did not deal with the principal mesenteric vessels which supply and drain the intestine, the connexions between these and the mesenteric microvasculature, or the relation between the type of vessel and its innervation.

MATERIALS AND METHODS

Tissue was taken from adult male and female rats which weighed 130–180 g. They were usually placed on a diet of cabbage plus water for 1–2 weeks prior to being killed. This regimen reduced the amount of mesenteric fat so that observation of the mesenteric microvasculature was easier.

The arrangement of the large vessels of the mesentery of the small intestine was examined under a dissecting microscope. In addition, several sets of vessels were excised and stained with 0·1 % Janus green B in 0·9 % NaCl. These preparations were dried on glass slides, washed in distilled water, dehydrated in alcohol, cleared and mounted. The topography of the mesenteric microvasculature was examined under the light microscope *in vivo* (Furness & Marshall, 1972), and in isolated preparations. Excised tissue was stretched on glass slides and allowed to dry. Some pieces were merely drained of blood and washed in saline. The vasculature of other preparations was filled with carmine gelatine (Moore, 1929) by the following procedure. The rats were killed by bleeding under ether anaesthesia, a centrally directed cannula was placed in the descending aorta just below the origin of the superior mesenteric artery, and a second cannula was placed in the hepatic portal vein to collect the outflow from the splanchnic region. The aorta, between the superior mesenteric and coeliac arteries, and the ileo-caeco-colic artery and vein were ligated. The mesenteric vessels were washed out with saline at 37 °C injected into the aortic cannula, and were then filled with carmine gelatine at 40 °C. This was allowed to cool to room temperature (about 18 °C) before areas of mesentery were removed. Animals were prepared in the same way for perfusion with Janus green B. The vessels were flushed out with saline, filled with the dye solution (0·1 % in saline) and left for 30 minutes. They were then washed through with saline until the effluent was clear of dye, perfused slowly with the mordant, 5 % ammonium molybdate, and washed again with saline.

In a few cases, the vessels were filled with carmine gelatine after staining with Janus green B. Pieces of mesentery were stretched on glass slides and allowed to dry over P_2O_5 in a desiccator. They were then fixed in formaldehyde vapour at 37 °C for 2 hours, washed with distilled water, dehydrated in alcohol, cleared in xylene, and mounted in dammar xylene.

Areas of mesentery were prepared as laminae for the demonstration of adrenergic nerves (Falck, 1962; Furness & Malmfors, 1971). Two counterstaining techniques were used with the fluorescence method, trypan blue for the blood vessels, especially the small veins, and neutral red for fat and lymphatics. Tissue was soaked in 0·1 % trypan blue in saline for 10–15 minutes at room temperature before being taken for the histochemical demonstration of adrenergic nerves. Rats were injected intravenously with 0·1 % neutral red, one hour prior to sacrifice. In order to ensure that even the faintest adrenergic nerves were observed, some preparations were loaded with noradrenaline. This was achieved by incubating pieces of mesentery in oxygenated Krebs's solution containing 10^{-7} g/ml noradrenaline tartrate and 10^{-4} g/ml pargyline hydrochloride for 30 minutes at 37 °C before they were dried. The relations between adrenergic nerves and mesenteric vessels were also examined in sections taken from paraffin-embedded tissue which had been freeze-dried under vacuum.

Dried laminae of the mesentery were stained for cholinesterases by the Koelle technique (Koelle & Friedenwald, 1949; Koelle, 1950) as modified by Lewis (1961) and Krnjevic & Silver (1965). For the demonstration of acetylcholinesterase (true cholinesterase; E.C. 3.1.1.7), the tissue was pre-incubated in 0·2 M-acetate buffer (pH 5·5) containing ethopropazine hydrochloride (10^{-4} M) for 30 minutes, and the same concentration of this inhibitor was also included in the incubation medium with the substrate, acetylthiocholine. The specificity of the reaction was tested by using butyrylthiocholine as a substrate with ethopropazine to inhibit 'pseudo' cholinesterase, and by using acetylthiocholine with ethopropazine and B.W. 284C51 (5×10^{-4} M) together as inhibitors. Incubation times were 5 and 20 hours at room temperature; the developer was 2% Na_2S, adjusted to pH 5·4 with acetic acid. Stretch preparations of the dilator muscle of the rat iris, a thin tissue known to contain cholinergic nerve terminals (Ehinger & Falck, 1966; Hökfelt, 1969), were similarly prepared and used as a comparison for evaluating the effectiveness with which cholinergic nerve fibres should be stained for acetylcholinesterase in the mesentery.

Some rats were injected with 6-hydroxydopamine hydrobromide (200 mg/kg, subcutaneous or intravenous), 48 hours before being killed, and tissue was taken for the histochemical demonstration of catecholamines and of cholinesterases.

RESULTS

The mesenteric vessels which are described supply the small intestine; the arteries arise from the superior mesenteric artery and the veins feed into the superior mesenteric vein (Fig. 1). The branching of the superior mesenteric artery in the rat is similar to that in other mammals (Grayson & Mendel, 1965). It gives rise to 1–2 duodenal branches and some 14–19 branches to the rest of the small intestine; it also provides a mid colic, a right colic and an ileo-colic branch (Fig. 1*a*). An additional small

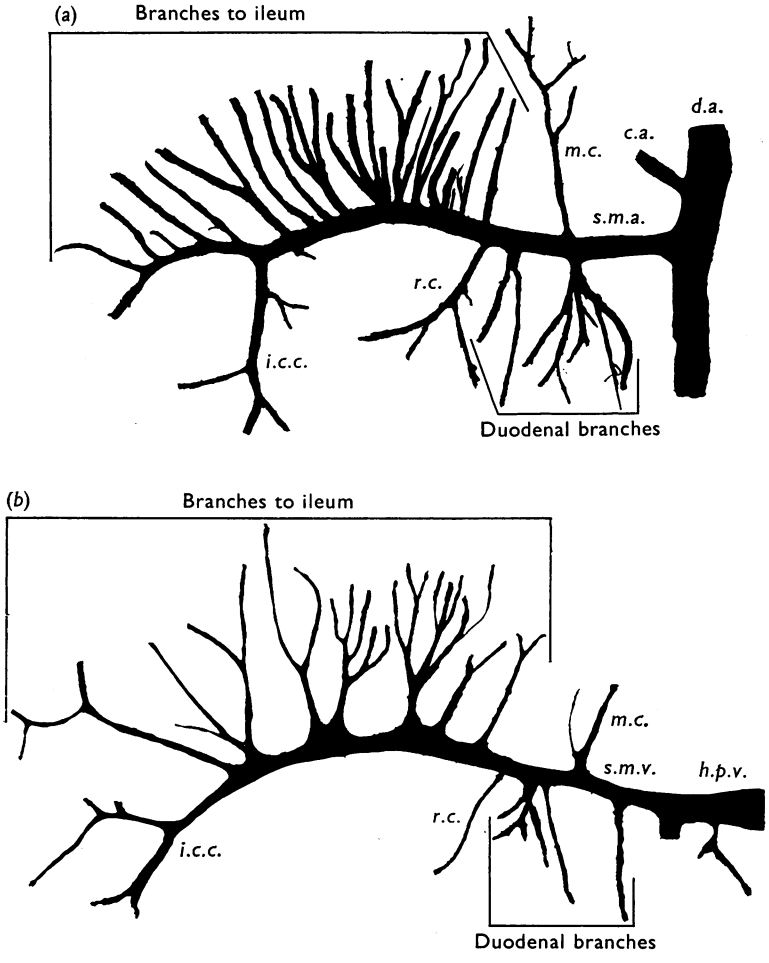

Fig. 1. The superior mesenteric artery (*s.m.a.*) and its principal branches (*a*). The superior mesenteric vein (*s.m.v.*) and its principal tributaries (*b*). These preparations were dissected from a rat of 160 g weight and stained with Janus green B. Each vessel has mid colic (*m.c.*), right colic (*r.c.*) and ileo-caeco-colic (*i.c.c.*) branches as well as branches to the ileum and duodenum. Note that there are more arterial branches to the ileum than there are venous branches. Coeliac artery: *c.a.* Descending aorta: *d.a.* Hepatic portal vein: *h.p.v.* The preparations were flattened against glass slides and so the diameters of the vessels appear greater than normal. Both $\times 2{\cdot}6$.

branch, which arises from the superior mesenteric artery between the right colic and the ileo-caeco-colic arteries, and supplies the proximal colon, has been observed in a few specimens. A branch of the mid colic artery follows the colon aborally and eventually anastomoses with a branch of the inferior mesenteric artery. The superior mesenteric vein is joined by tributaries corresponding to the duodenal and colic arteries, but there are fewer veins (7–10) joining the superior mesenteric vein from the rest of the small intestine than there are arteries entering this area. It is notable that these tributaries are often formed by the junction of several mesenteric veins within 1–2 mm of the superior mesenteric vein (Fig. 1*b*). Each mesenteric artery

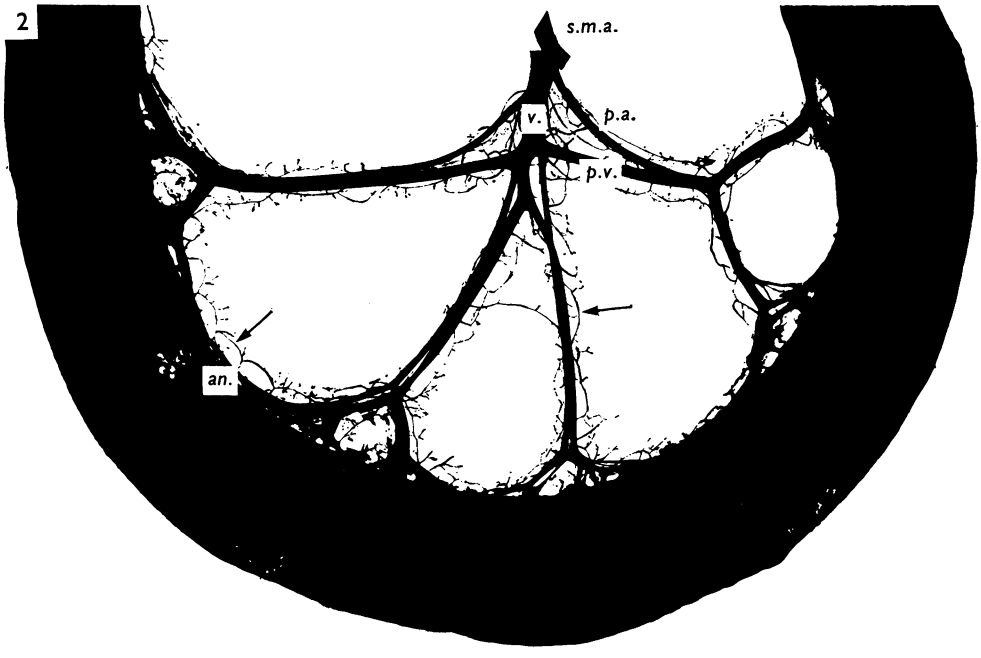

Fig. 2. The blood vessels of the mesentery to the small intestine, stained with Janus green B and filled with carmine gelatine. The principal mesenteric arteries (*p.a.*), principal mesenteric veins (*p.v.*) and small arteries of the mesenteric microvasculature (arrows) can be seen. The principal arteries leave the superior mesenteric artery (*s.m.a.*) separately, but the principal veins join to form a common vessel (*v.*) before entering the superior mesenteric vein (not shown). There are arterial and venous anastomoses (*an.*) close to the gut wall between successive groups of vessels. Note that the small arteries form loops. × 2·5.

branches 2–3 times before coming close to the wall of the intestine where the vasa recta arise (Fig. 2). Branches from adjacent mesenteric arteries form anastomoses close to the gut wall. The veins follow the arteries and join where the latter branch, except that the veins often combine immediately before entering the superior mesenteric vein whereas the arteries arise separately from the superior mesenteric artery (Fig. 2). This simple pattern of branching of the principal mesenteric vessels is very similar to that in the dog (Eisberg, 1924; Noer, 1943). It is different from the human (Cokkinis, 1930), in that, in the rat, the vasa recta are much shorter and series of cross-anastomoses forming prominent arcades in the mesentery are not found.

The topography of the microvasculature

Fig. 3 has been drawn from a specimen injected with carmine gelatine to demonstrate the relationship between the principal mesenteric vessels and the microvasculature and to show the nomenclature which has been adopted in the present paper. The first components of the microvasculature were the small arteries which formed loops, 1–5 mm long, and were joined at both ends to principal mesenteric arteries (Fig. 3). A short length of small artery was usually common to adjacent loops. There were two chains of loops, one each side, associated with each principal mesenteric

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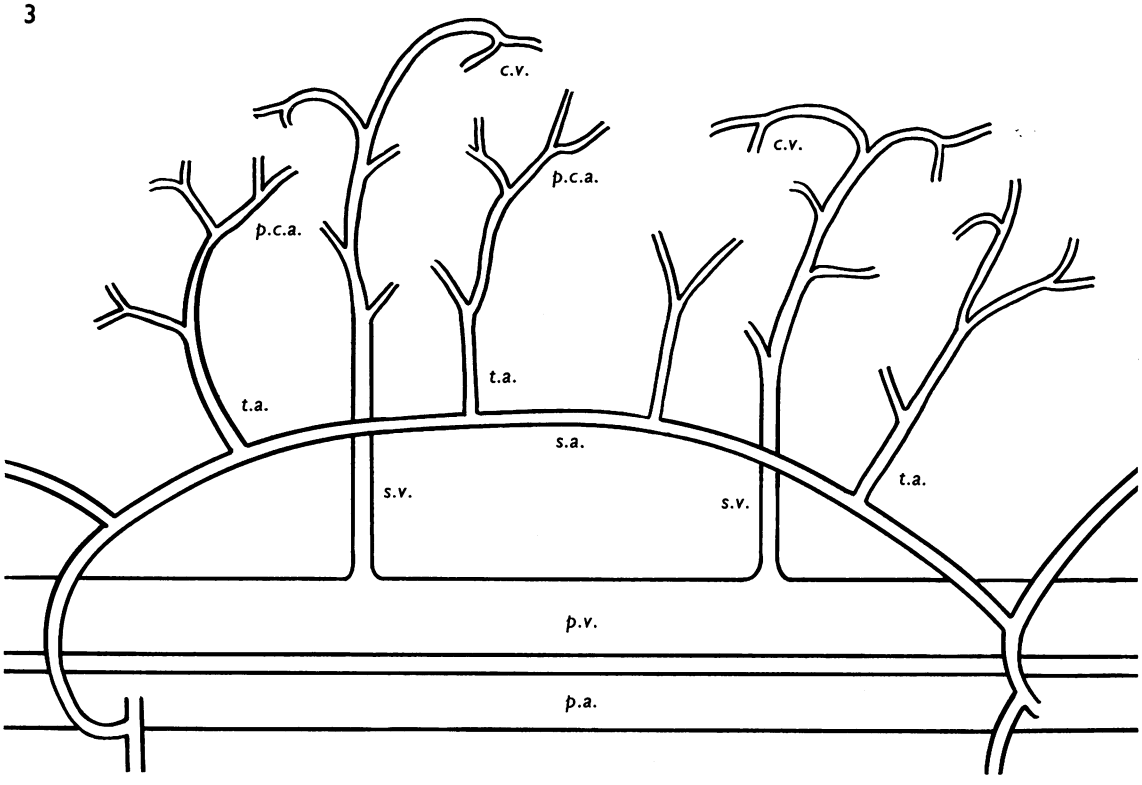
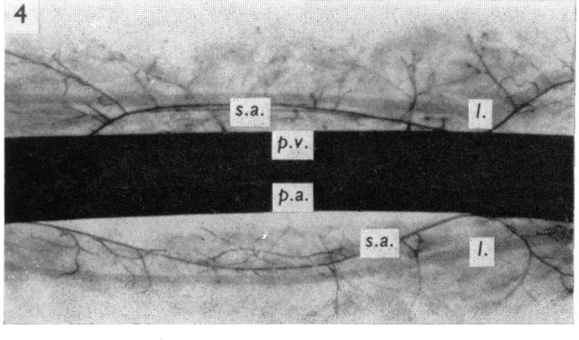




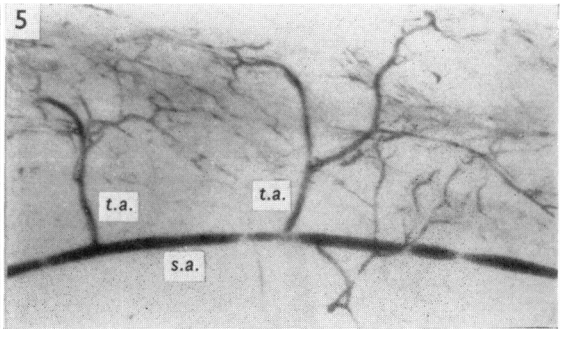


Fig. 3. A simplified drawing from an area of the mesenteric vasculature filled with carmine gelatine to show the nomenclature used in the present work and to demonstrate the relationship between the principal mesenteric vessels and the mesenteric microvasculature. Principal artery: *p.a.* Principal vein: *p.v.* The main purpose of these vessels is to supply and drain the intestine, but they also give rise to the mesenteric microvasculature. Small artery: *s.a.*; these vessels form short loops in parallel with the principal arteries. Terminal arterioles: *t.a.* Precapillary arterioles: *p.c.a.* The transition between terminal and precapillary arterioles is discussed in the text. Collecting venules: *c.v.*; small veins: *s.v.* Approximately × 50.

Fig. 4. The appearance of an area of the mesenteric vasculature in a specimen stained with Janus green B and injected with carmine gelatine. Only the arterial side of the microvasculature is filled. Labelling as in Fig. 3. Lymph vessels (*l.*) and the mesenteric fat have been stained lightly. × 15.

Fig. 5. Part of a specimen injected with carmine gelatine. The manner in which the terminal arterioles branch from the small arteries and the relationship of precapillary arterioles to terminal arterioles can be seen. Compare with Fig. 3. × 30.

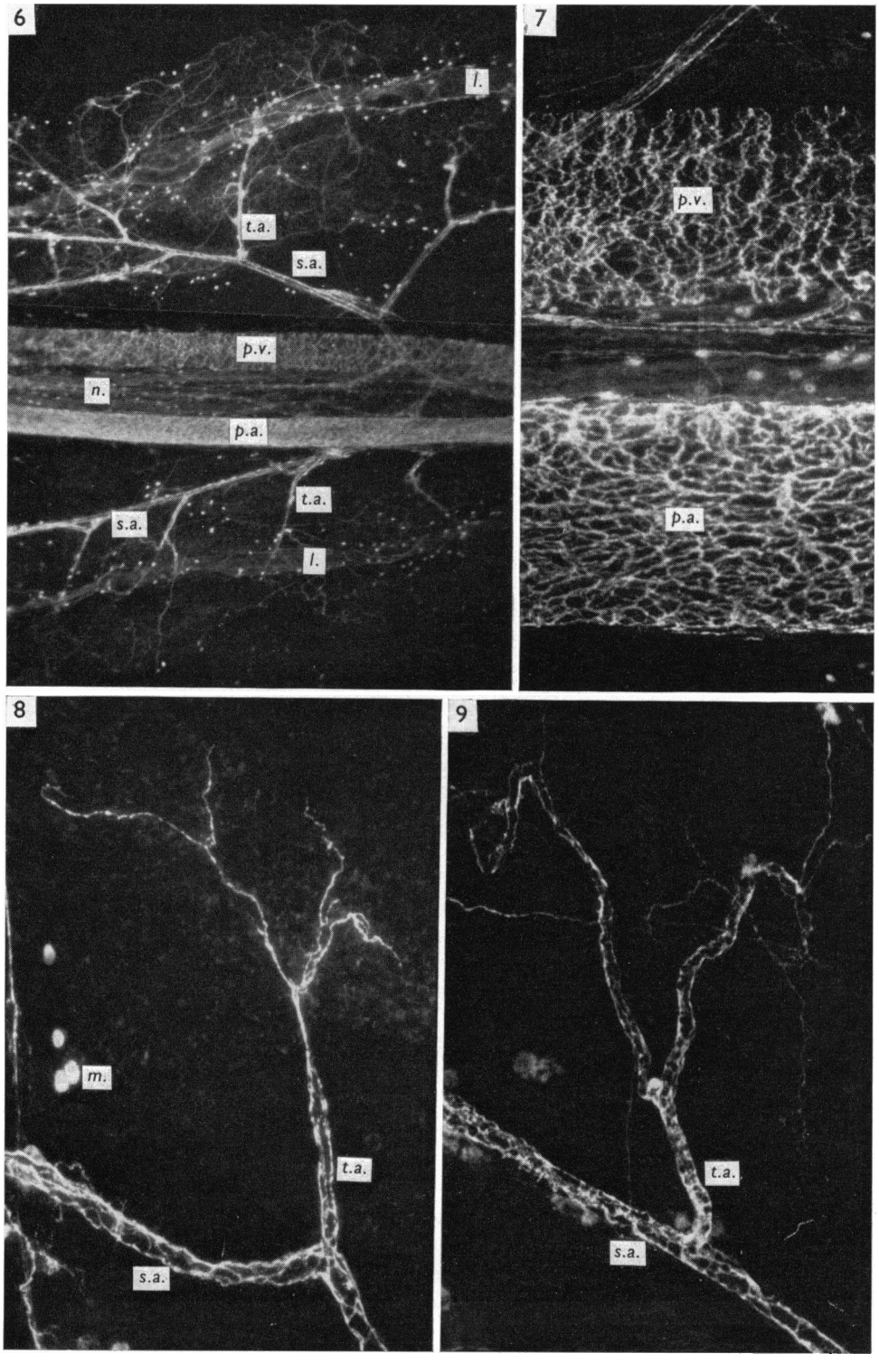
artery (Figs. 2, 4), but there was only one series of small loops, on the side away from the intestine, with each of the arterial anastomoses lying close to the gut wall (Fig. 2). Internal diameters of small arteries, measured *in vivo*, were 20–40 μm. Each small artery gave rise to a series of terminal arterioles, 18–30 μm in internal diameter (Figs. 3, 5). The definition of terminal arterioles used in the present paper is that of Zweifach (1961). The terminal arterioles did not form anastomoses with each other and seldom gave rise to capillaries. The terminal arterioles became narrower, and usually branched to form the precapillary arterioles, which were 10–20 μm in internal diameter *in vivo*. A precise transition between the terminal arterioles and the precapillary arterioles cannot be defined. Nevertheless, in terms of their innervation and reactions to stimuli (dealt with below) some distinction needs to be made. Zweifach (1961) has referred to the muscular vessels beyond the terminal arterioles as metarterioles and the junctional segments between these and their capillary offshoots as precapillary sphincters. In the present work the term precapillary arteriole, also mentioned by Zweifach, is intended to include both the metarterioles and the precapillary sphincters. Examples of the relations between small arteries and their branches are shown in Figs. 4 and 5, which are micrographs taken from preparations filled on the arterial side with carmine gelatine.

As implied above, it was the precapillary arterioles which gave rise to the capillaries. These were 3–10 μm in internal diameter *in vivo*. The capillaries joined to form collecting venules (12–30 μm in internal diameter) which in turn combined to form small veins, 20–50 μm in diameter, and these entered the principal mesenteric veins (Fig. 3). The small veins, unlike the small arteries, usually did not form loops, although in some instances connexions between tributaries of adjacent small veins were traced. The branching of the capillaries from the precapillary arterioles, the general pattern of the capillaries, and their confluence with the collecting venules has been described in detail previously (Chambers & Zweifach, 1944; Zweifach, 1961); the present observations concur with their descriptions.

The pattern of the mesenteric microvasculature outlined above applies to most of this system, that is, the vessels supplying the mesenteric fat. In a few places, small systems of vessels thrust further into the mesentery, beyond the strips of fat following the principal vessels. Some of these vessels formed simple systems, such as Chambers & Zweifach (1944) described. A lymphatic vessel sometimes followed an independent course through the mesentery and was accompanied by a small artery, small vein and simple microvascular system. Most lymphatic channels were in the fat close to the principal vessels.

Adrenergic nerves

The fluorescence histochemical method (Falck, 1962) was used to examine the distribution of adrenergic nerves in air-dried laminae of the mesentery. The fluorescence in the nerves was abolished in animals treated 48 hours before death with 6-hydroxydopamine (200 mg/kg i.v. or s.c.). The reaction was also absent from fibres peripheral to an interruption of the paravascular nerves by crushing (see Furness & Costa, 1971), 3 or 4 days prior to death. The present method probably revealed all the adrenergic terminals, because there was no difference in the distribution of fibres between normal preparations and tissue which had been loaded with noradrenaline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(10^{-7} g/ml) in the presence of the monoamine oxidase inhibitor, pargyline hydrochloride (10^{-4} g/ml). The loading was for 30 minutes in oxygenated Krebs's solution at 37 °C.

A low power view of the adrenergic innervation of mesenteric vessels is shown in Fig. 6. It can be seen that the principal artery and vein and the small arteries and terminal arterioles are all innervated, but that few nerves are associated with other elements in the mesentery. The many mast cells, which have a yellow fluorescence, appear as white dots in this and subsequent figures. The adrenergic nerves form dense networks around the principal arteries and veins (Fig. 7) and around the superior mesenteric artery and vein. The fluorescent fibres were not aligned with the smooth muscle cells which form bundles encircling the arteries, but they created a close meshwork at the surface of the medial smooth muscle. The fibres innervating the principal veins appeared finer than those supplying the arteries, and many ran parallel to the circularly disposed smooth muscle. Transverse sections of the vessels showed that most of the adrenergic terminals lay at the outer limits of the smooth muscle coats of both arteries and veins (for an illustration of this point see Furness, 1971). The principal mesenteric vessels were accompanied by paravascular nerve trunks containing non-varicose fluorescent axons.

The small arteries and the terminal arterioles of the mesenteric microvasculature were supplied by adrenergic axons which ran along and crossed over them, forming a loose network (Figs. 8, 9). In a few instances, a small artery was followed by a fine nerve trunk, separated from the vessel wall by about 5–10 μm. No specialization in the innervation could be recognized at the points where the small arteries branched from the principal mesenteric arteries or where the terminal arterioles branched from the small arteries. Nerve fibres could be traced from the perivascular plexus of the parent vessel (principal artery or small artery) to the plexus of its branch (small artery or terminal arteriole). The perivascular networks petered out in the area of transition from terminal arteriole to precapillary arteriole. Some axons ended in this transitional zone and others ran into the fat, sometimes first following a precapillary arteriole or a true capillary for a short distance. The fairly abrupt ending of the network of adrenergic nerves, and the continuation of the precapillary arteriole beyond this point were observed clearly by comparing the same area, illuminated with ultraviolet light to reveal the adrenergic nerves, and illuminated with white light using phase contrast optics to show the blood vessels (Figs. 10, 11). The first few micrometres of the capillaries after they branched from the arterioles, that is, the

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Fig. 6. A low power view of the fluorescence appearance of adrenergic nerves in the rat mesentery. Labelling of the vessels as in Fig. 3. Lymphatics: *l.* Paravascular nerve trunks: *n.* The many small white dots are mast cells. Note that adrenergic terminals supply the principal artery and vein and the small arteries and terminal arterioles. × 25.

Fig. 7. The adrenergic innervation of the principal mesenteric vessels. Principal artery: *p.a.* Principal vein: *p.v.* × 95.

Figs. 8 and 9. Adrenergic nerves with the small arteries (*s.a.*) and terminal arterioles (*t.a.*) of the mesenteric microvasculature. Note that the plexuses of the small arteries are continuous with those of the terminal arterioles, but there are no apparent specializations at the points of branching. The innervation follows the terminal arterioles and then peters out. Mast cells: *m.* The fat can be seen faintly in the background to Fig. 8. Both × 150.

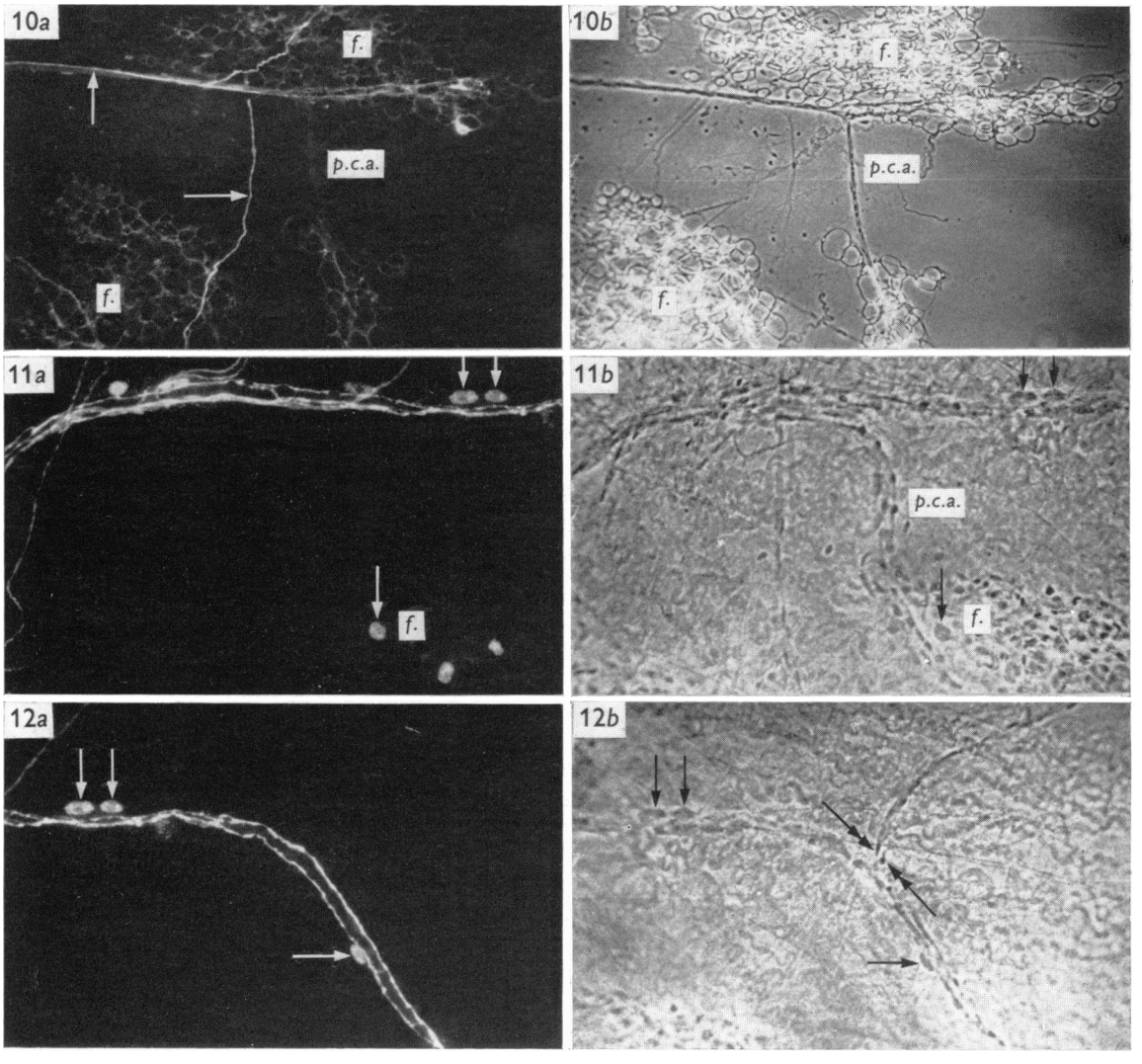

Fig. 10. The same area photographed under ultraviolet illumination (*a*) to show adrenergic nerves (arrows) and under white light with phase contrast optics to show blood vessels (*b*). The specimen was counterstained with neutral red. The precapillary arteriole (*p.c.a.*) is not innervated. Note that the adrenergic axon indicated by the horizontal arrow does not supply a blood vessel or the fat (*f*). × 50.

Fig. 11. Adrenergic nerves (*a*) and the background tissue (*b*): fluorescence and phase contrast microscopy of the same area. Note that the precapillary arteriole (*p.c.a.*) is not innervated. Arrows point to mast cells which are visible in both micrographs. Fat: *f*. × 120.

Fig. 12. Part of the mesentery shown by fluorescence (*a*) and phase contrast (*b*) microscopy. A precapillary sphincter, which is not innervated by adrenergic nerves, is indicated by the double arrows. Single arrows point to mast cells. × 120.

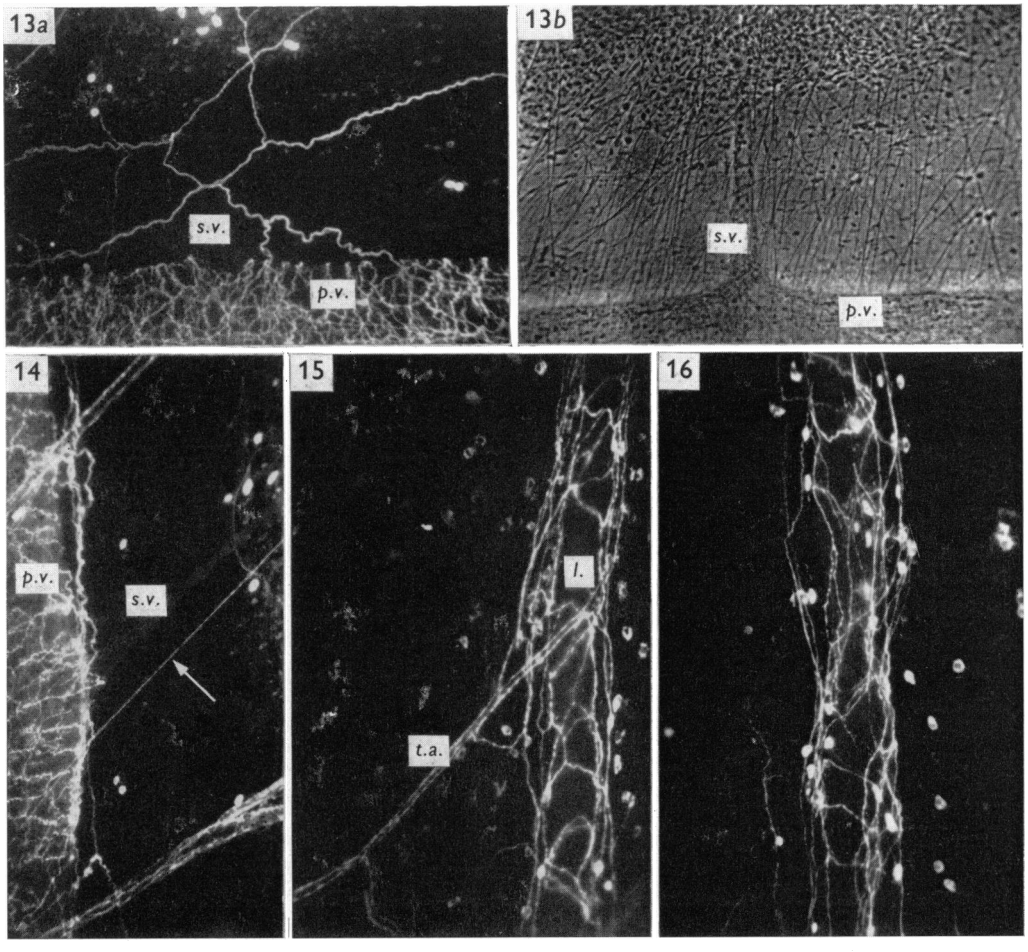

Fig. 13. Principal mesenteric vein (*p.v.*) and a tributary (*s.v.*) shown under fluorescence illumination (*a*) and with phase contrast optics (*b*). The tributary is uninnervated. × 60.

Fig. 14. From a specimen stained with trypan blue and then prepared for the demonstration of adrenergic nerves. A small vein (*s.v.*) which joins the principal vein (*p.v.*) is followed by an adrenergic fibre (arrow), but is not itself innervated. × 60.

Fig. 15. Adrenergic fibres supplying a terminal arteriole (*t.a.*) and a lymphatic vessel (*l.*). Note that fibres in the plexus of the arteriole are continuous with those supplying the lymphatic. × 60.

Fig. 16. The adrenergic innervation of a lymphatic. Preparation lightly stained with neutral red to show the vessel wall. Note the frequent mast cells close to the lymphatic. × 60.

regions of the precapillary sphincters, were not innervated (Fig. 12). In a few cases, a single fibre left the periarterial network and followed a capillary, but in no instances were fibres seen to wrap around the precapillary arterioles or capillaries or to form anything resembling a network such as invested the small arteries and the terminal arterioles.

There were no perivascular networks associated with the collecting venules, and adrenergic fibres were rarely seen to lie close to any of these vessels. Most of the

small veins which joined the principal mesenteric veins were similarly bereft of an adrenergic innervation (Figs. 13, 14). Occasionally, a single fibre branched from the perivascular plexus surrounding a principal vein and followed a small vein into the fat (Fig. 14).

Fine fluorescent fibres were associated with the main lymph vessels (Figs. 15, 16). These fibres ran along and crossed over the lymphatics, forming an open meshwork. Some of the fibres were often several micrometres from the wall of the lymphatic. In many places nerve fibres which followed arterioles continued as part of the plexuses around the lymphatics (Fig. 15); in fact it seemed likely from the observations that nearly all the adrenergic fibres accompanying the lymphatics were extensions of periarterial nerves. Innervation of the valves of the lymph vessels was never observed. An adrenergic innervation of the lymphatics in the rat mesentery accords with the observation (Florey, 1927) that these vessels are excited by stimulation of the splanchnic nerves and by adrenaline.

In all preparations there were some fine fibres which appeared to innervate neither blood vessels, nor fat, nor lymphatic vessels. Some of these ran near, but separate from, the principal mesenteric vessels (Fig. 13) and others were found in the mesentery in areas in which only squamous cells and connective tissue could be identified in the background by phase contrast microscopy. These latter fibres were nearly all within a few millimetres of the intestine; they may form part of the serous plexus which has been described lying close to the gut wall particularly in the region of its mesenteric attachment (Schofield, 1968), and has been shown to contain adrenergic fibres in the region of the stomach (Furness, 1971).

Localization of acetylcholinesterase

Pieces of mesentery which were incubated with acetylthiocholine as substrate and ethopropazine as inhibitor showed heavy deposits of stain on the paravascular nerves, very weak or no stain in the plexuses of the principal mesenteric vessels, and no staining of the nerves supplying the mesenteric microvasculature after 5 hours. After a 20 hour incubation, there was strong staining of the paravascular nerves and moderate staining of fibres in the perivascular plexuses of the principal mesenteric vessels and around the small arteries and terminal arterioles (Figs. 17, 18). The distribution of these fibres was remarkably similar to that of adrenergic fibres (described above). In the iris, terminal fibres had a moderate to strong stain after 5 hours and were heavily stained at 20 hours. No staining of nerves in the mesentery or the iris was observed if B.W.284C51 as well as ethopropazine was used as an inhibitor with acetylthiocholine as substrate. When butyrylthiocholine was used as a substrate, with ethopropazine as inhibitor, there was very weak staining of the paravascular nerves, but no staining of the perivascular plexuses after 20 hours. The enzyme localized with fibres in the perivascular plexuses of mesenteric vessels is characterized as acetylcholinesterase (E.C. 3.1.1.7) because of its selective hydrolysis of acetylthiocholine, its resistance to ethopropazine and its susceptibility to inhibition by B.W.284C51 (Koelle, 1955; Bayliss & Todrick, 1956; Holmstedt, 1957; Naik, 1963). These results indicate that acetylcholinesterase is associated with fibres having the same distribution as adrenergic terminals in the mesentery and that the activity of the enzyme is less than that seen in known cholinergic terminals of the

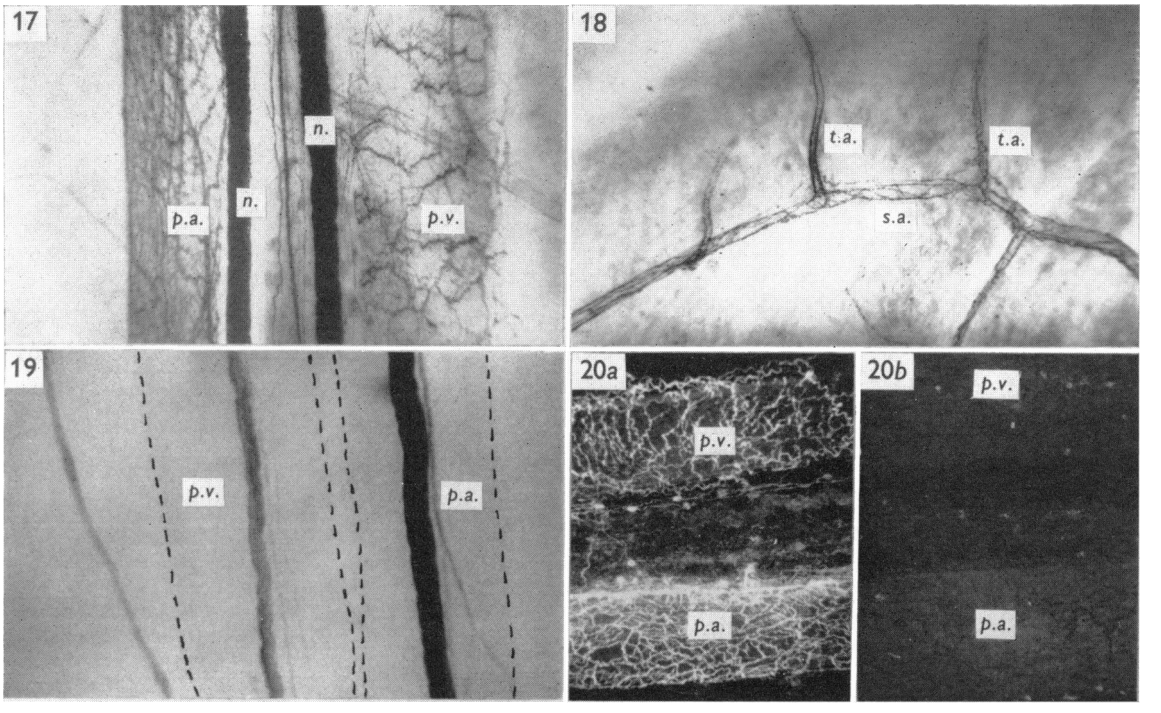

Fig. 17. The localization of acetylcholinesterase with a principal artery (*p.a.*) and principal vein (*p.v.*) in the mesentery. Incubated for 20 hours with acetylthiocholine as substrate. Ethopropazine used to inhibit other cholinesterases. The paravascular nerves (*n.*) and the perivascular plexuses are stained. × 70.

Fig. 18. Acetylcholinesterase-positive nerves associated with a small artery (*s.a.*) and with terminal arterioles (*t.a.*) of the mesenteric microvasculature. Treatment as for Fig. 17. × 70.

Fig. 19. The localization of acetylcholinesterase with mesenteric vessels from a rat pre-treated by an injection of 6-hydroxydopamine 2 days before it was killed. This preparation was incubated in the same container as the preparation shown in Fig. 17. Note that paravascular nerves are still heavily stained but that terminal plexuses associated with the principal artery (*p.a.*) and vein (*p.v.*) are no longer positively stained for acetylcholinesterase. The boundaries of the vessels were determined by examination with phase contrast optics and have been drawn in. × 70.

Fig. 20. A control preparation (*a*) and a preparation from the rat pretreated with 6-hydroxydopamine (*b*) which was used to make Fig. 19. Fluorescent adrenergic nerves, present in the control, are absent after the injection of 6-hydroxydopamine. The two specimens were prepared together for fluorescence microscopy. The positions of the principal artery (*p.a.*) and vein (*p.v.*) in the micrograph from the treated animal can be seen by their slight non-specific fluorescence. Both × 60.

iris. To test the possibility that, in the mesentery, acetylcholinesterase was associated with adrenergic fibres, rats were injected with 6-hydroxydopamine, which causes a selective degeneration of adrenergic nerve terminals (Malmfors & Thoenen, 1971). In tissue taken two days after this treatment, there was an almost complete abolition of staining of the perivascular plexuses for acetylcholinesterase (Fig. 19), although the staining of the paravascular nerve trunks was not changed significantly. The fine fibres associated with the small arteries and arterioles of the microvasculature were no longer stained. Most of the fibres innervating the principal veins were now un-

stained, and in those that were positive, staining was extremely faint. In most places the plexus around the principal arteries had disappeared, but a few areas of weakly stained fibres were encountered. There was also an almost complete disappearance of the adrenergic terminals demonstrable by the fluorescence histochemical method (Fig. 20). Some parts of the paravascular nerve trunks still showed fluorescence.

DISCUSSION

The present experiments indicate that the small arteries and terminal arterioles of the mesenteric microvasculature receive an adrenergic innervation, but that, although single adrenergic fibres sometimes follow precapillary arterioles (metarterioles and precapillary sphincters), these vessels are usually not innervated. The distribution of adrenergic nerves is consistent with the responses of the vessels to nerve stimulation, which causes constriction of the small arteries and terminal arterioles but does not affect the precapillary arterioles (Furness & Marshall, 1973*a*, *b*). The close correlation between the presence (or absence) of a periarterial network of adrenergic nerves and the presence (or absence) of a response to nerve stimulation, and the fact that the precapillary arterioles are extremely sensitive to noradrenaline (Altura, 1971*a*, *b*), indicate that noradrenaline released in response to nerve stimulation at low frequencies (less than 6 Hz; Furness & Marshall, 1973*a*, *b*) does not diffuse in any significant concentration to the precapillary arterioles. Adrenergic axons which followed precapillary arterioles, but were separated from the vessel wall by several micrometres, and also axons which, in following a parent vessel, came close to a precapillary branch (e.g. Fig. 12) were encountered in the present work, and it is possible that with high rates of stimulation sufficient noradrenaline could diffuse from these axons to influence otherwise uninnervated vessels. The precapillary arterioles are very sensitive to a number of substances in addition to noradrenaline, and it is probable that their diameters are influenced by factors, including noradrenaline, in the circulation, and also by the local release of tissue metabolites (Zweifach, 1961; Altura, 1971*b*). The dense adrenergic innervation of the superior mesenteric artery and the principal mesenteric arteries suggests that these larger vessels play an important part in the control of blood flow to the intestine and mesenteric microvasculature (see also Furness, 1971; Furness & Marshall, 1973*b*).

Most of the blood in the splanchnic region is contained in the veins (Lacroix, 1960; Bradley, 1963) and some 35–40% of this blood can be displaced from the splanchnic bed by moderate stimuli applied to vasoconstrictor nerves (Folkow *et al.* 1964). The collecting venules and the small veins of the mesenteric microvasculature, as well as the veins within the gut wall (Furness, 1971; Furness & Costa, 1973) are very sparsely, if at all, innervated, whereas larger veins (principal mesenteric, superior mesenteric and hepatic portal) are supplied by dense plexuses of adrenergic nerves. Thus it would seem that it is the constriction of the larger veins which contributes most to the nerve-mediated diversion of blood volume from the splanchnic region.

Previous ultrastructural and histochemical studies have shown that arterioles and medium-sized arteries receive an adrenergic innervation (see Introduction), but, except for the work of Samarasinghe (1965) and Rhodin (1967), they do not indicate whether this innervation extends to the precapillary arterioles. However, there is

some basis for believing that lack of innervation of precapillary arterioles, as reported in the present work, might apply in other species and other vascular beds. Sandison (1932), who made observations on blood vessels in the rabbit ear, found that small arteries contracted in response to cold but that arterioles and venules did not. All the vessels were constricted by adrenaline. If it is assumed that the constriction in response to cold resulted from the activation of sympathetic (adrenergic) nerves, as seems certain (Ström, 1960; Greenfield, 1963), then the situation is similar to that in the mesentery, the innervation ending before the capillary bed is reached and being absent from the collecting venules. Clark, Clark & Williams (1934) reported experiments which support this interpretation. By using methylene blue to stain nerve fibres *in vivo* they found that newly formed vessels in the rabbit ear became partly innervated, leaving many arterioles without a nerve supply. The microvasculature of a skeletal muscle, the tenuissimus of the cat, has similar properties to that of the rat mesentery; when vasoconstrictor nerves are stimulated, arterioles of diameters greater than 15 μm respond, but the smaller vessels are unaffected (Eriksson & Lisander, 1972). However, the situation appears to be somewhat different in the fascia of rabbit thigh muscle. Rhodin (1967) made a detailed ultrastructural study of vessels in this tissue and found that the innervation continued to the precapillary sphincters, which were 7–15 μm in diameter. The sphincter areas, and the arterioles from which they branched, often had several nerve fibres associated with them, and the presence of small granular vesicles within some of the axons suggests that an adrenergic innervation continues further along the arterial tree in the rabbit fascia than it does in the rat mesentery. Ultrastructural details of the innervation of rat cerebral arteries have been reported by Samarasinghe (1965), who found that the major extracerebral arteries were innervated, but that their intracerebral extensions as well as extracerebral arterioles (outside diameter 10–20 μm) were not. I have examined the mesenteries of cat, rabbit and guinea-pig (Furness, 1971), and, in rather less extensive studies of these species, a pattern of innervation of the mesenteric microvasculature similar to that in the rat was observed. Hence it may be speculated that the pattern of innervation observed in the rat mesentery is repeated in at least some other vascular beds, and that nervous control over blood flow in these tissues is exerted on medium-sized and small arteries but not on the precapillary arterioles.

The responses of the arteries and arterioles of the rat mesentery to nerve stimulation seem to be mediated entirely through the activation of adrenergic axons, in that they are abolished by the adrenergic neuron-blocking drugs, guanethidine and bretylium, by alpha-adrenoceptor antagonists and by pretreatment with reserpine (McGregor, 1965; Malik & Ling, 1969; Furness & Marshall, 1973*b*). The present histochemical results are in accord with those pharmacological observations; an adrenergic innervation was demonstrated by the fluorescence histochemical method, but acetylcholinesterase activity in the fibres was weak, and was abolished in animals pretreated with 6-hydroxydopamine, a drug which causes the selective degeneration of adrenergic nerves (Malmfors & Thoenen, 1971). Ultrastructural studies indicate that all the axons innervating mesenteric vessels in the rat contain small granular vesicles, and are thus probably adrenergic (Devine & Simpson, 1967).

SUMMARY

The principal arteries of the mesentery of the small intestine in the rat arise as 14–19 branches of the superior mesenteric artery. In addition to supplying the intestine, they give rise to a series of small arteries which are the first components of the mesenteric microvasculature. Branching from the small arteries are the terminal arterioles, which in turn provide the precapillary arterioles (metarterioles and precapillary sphincters). The microvasculature drains into the principal mesenteric veins through collecting venules and small veins.

Networks of adrenergic axons innervate the superior mesenteric artery and vein, the principal arteries and veins, the small arteries and the terminal arterioles. The precapillary arterioles (although they possess muscular walls), the collecting venules, and the small veins, are not innervated. Low levels of acetylcholinesterase are associated with the adrenergic nerves, but evidence is presented which indicates that there is no cholinergic innervation of the mesenteric blood vessels. There is a sparse adrenergic innervation of lymphatic vessels in the mesentery; the fibres supplying these vessels are continuous with fibres of the plexuses around terminal arterioles.

It is concluded that the neural control of blood flow through the mesenteric microvasculature is mediated by adrenergic nerves which constrict the principal arteries, small arteries and terminal arterioles. Precapillary arterioles are not directly influenced by nerves, but are probably under humoral control. Adrenergic nerves which modulate blood volume in the splanchnic region constrict large veins, but not small veins or venules.

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